

# 09. (September) 2019

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**Project:** iGEM\_Munich2019 Shared Project

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## RNA extraction from VLPs

- SN spun down 10min, 2000g
  - 400µl used for RNA extraction using MagMAX viral RNA isolation kit
  - manufacturer's instructions were followed
  - elution was done in 25µl Nuclease-free water at 50°C for 4min
  - samples frozen at -20°C
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- Cells RNA extracted with Qiagen's RNeasy Plus Mini Kit
  - 400µl lysis buffer used per well
  - RNA eluted in 40µl nuclease free water
  - samples frozen at -20°C
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- Exosomes received (condition 1 + 3-6; SN volume: 1500 µl (2x750µl Eppis) of 1,3,4,5; 400 µl of condition 6)
  - incubated overnight with Exosome isolation reagent ( 375 µl per eppi[to 750µl SN]; 200 µl condition6 [to 400µl SN]) + 1µl Spike per condition

## RT of 48h Exo SN1

- 2x RT & noRT
- primer RT3

## PCR

- samples 2x SN1 RT, SN1 noRT, 1x mock RT
- Q5 Master Mix
- per sample: 12.5µl Q5 MM
  - 1.25µl each primer 41 & 42
  - 9µl ddH2O
  - 1µl Template

Annealing 67°C